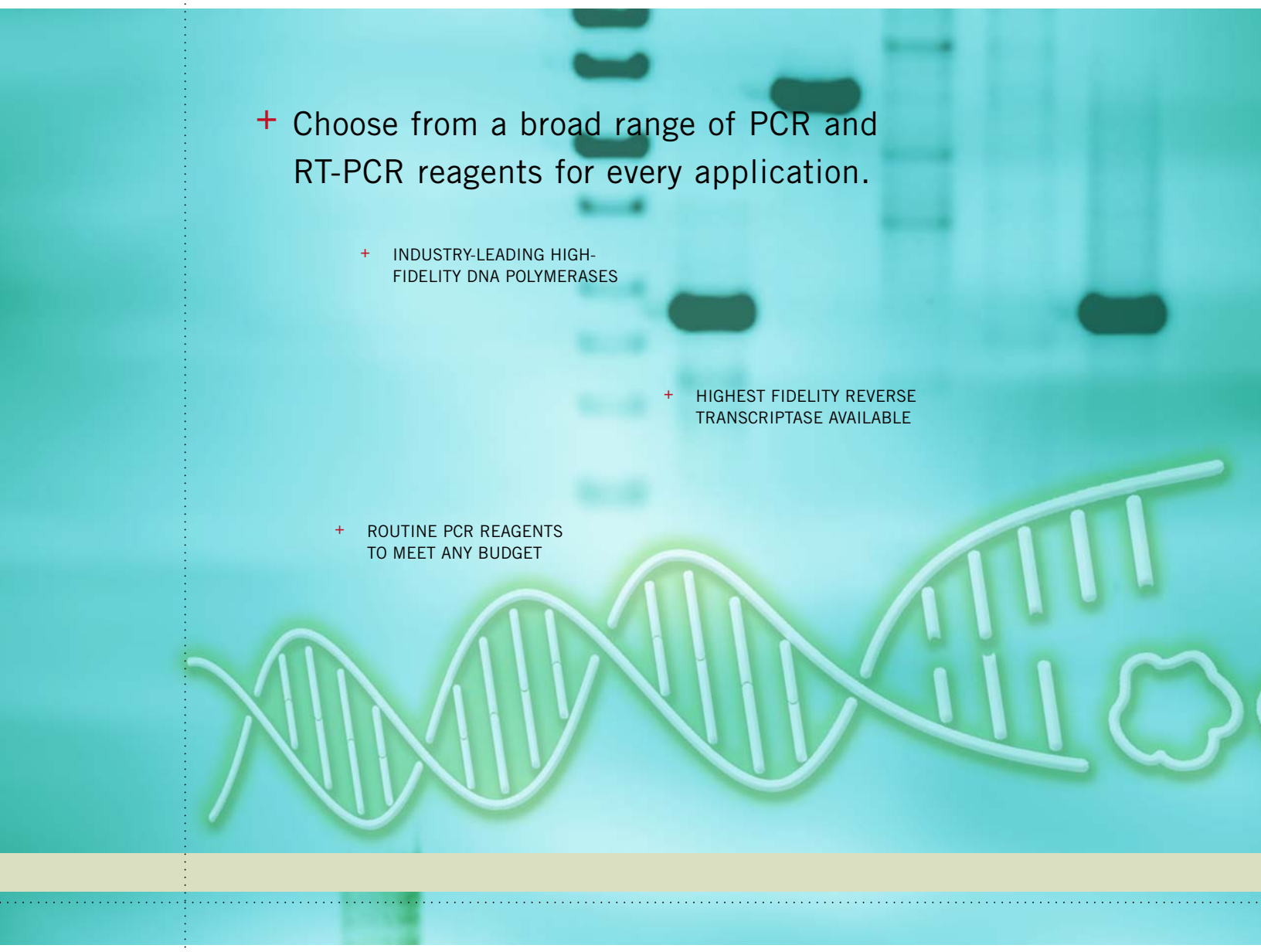
AMPLIFICATION

PCR and RT-PCR Reagents



+ Choose from a broad range of PCR and RT-PCR reagents for every application.

+ INDUSTRY-LEADING HIGH-FIDELITY DNA POLYMERASES

+ HIGHEST FIDELITY REVERSE TRANSCRIPTASE AVAILABLE

+ ROUTINE PCR REAGENTS TO MEET ANY BUDGET

STRATAGENE
An **Agilent Technologies** Company

We have the best PCR and RT-PCR reagents for your application.

Our expertise in enzyme engineering has produced one of the broadest portfolios of high-fidelity PCR enzymes and reverse transcriptases available today. This expertise has translated into advances in high-fidelity PCR and reverse transcription.

Whether you need the highest accuracy available, large product yields, or sensitive cDNA synthesis, trust our high-performance enzymes to address your amplification challenges.

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The Importance of High Fidelity

High-fidelity PCR enzymes are valuable for minimizing the introduction of amplification-generated errors in products that will be cloned, sequenced, and expressed. Employing high fidelity amplification procedures can save significant time and effort, and may minimize the number of clones that must be sequenced to obtain error-free constructs or accurate consensus sequences. Moreover, the use of high fidelity amplification is essential when analyzing small amounts of template DNA or rare molecules in heterogeneous populations. Amplicons generated from small amounts of template DNA are especially prone to high mutation frequencies because PCR-generated errors in early cycles are further amplified in each subsequent cycle.[1,2]

The ArchaeMaxx® Factor Advantage

As proofreading PCR enzymes can be challenging to optimize, we have improved high fidelity PCR enzymes so that they are more reliable. Found exclusively in our high fidelity DNA polymerases, our ArchaeMaxx® Polymerase-Enhancing Factor improves overall PCR performance by eliminating the inhibition of proofreading enzymes caused by incorporation of dUTP, which results from deamination of dCTP during PCR[3]. Our PCR enzymes formulated with the ArchaeMaxx factor produce higher yields and exhibit greater target length capability.

Superior Reverse Transcription

Our expertise in enzyme engineering also extends to reverse transcriptases (RT), and we have developed the most accurate reverse transciptases available. Our AccuScript® High-Fidelity Reverse Transcriptase allows more efficient and accurate production of full-length cDNA. In addition, our AffinityScript™ Multiple Temperature Reverse Transcriptase allows you to reverse transcribe at your preferred reaction temperatures.

High-Fidelity PCR Enzymes

High-Fidelity PCR Enzymes	Application	Accuracy	Reliability
PfuUltra® II Fusion HS DNA Polymerase[a]	Ultra-High Fidelity Cloning and Mutagenesis	★★★★★	★★★★★
PfuUltra® High-Fidelity DNA Polymerase[a]	Ultra-High Fidelity Cloning and Mutagenesis	★★★★	★★★
PfuTurbo® DNA Polymerase[a]	High-Fidelity Cloning and Mutagenesis	★★★	★★★
Pfu DNA Polymerase, cloned[b] or native[c]	High-Fidelity Cloning and Mutagenesis	★★★	★
Herculase® II Fusion DNA Polymerase[a]	Superior Yield / Routine Cloning/ GC-Rich Targets	★★★	★★★★★
Easy-A® High-Fidelity PCR Cloning Enzyme[d]	High-Fidelity TA / UA Cloning	★★★	★★★
EXL® DNA Polymerase[e]	Amplifying Extra-Long Targets	★★	★★★
PicoMaxx® High-Fidelity PCR System[e]	Sensitivity	★★	★★★

MUTATION FREQUENCIES OF DNA POLYMERASES

PCR Enzyme	Error Rate	Accuracy (error rate^{-1} in bases)	Mutation Frequency*	% of Clones with Mutations	Number of Mutant Clones per 200 Colonies
PfuUltra® II	4.00×10^{-7}	2,500,000	0.0064	0.64%	1
PfuUltra®	4.44×10^{-7}	2,250,000	0.0071	0.71%	1
PfuTurbo®	1.33×10^{-6}	750,000	0.0213	2.13%	4
Pfu cloned or native	1.33×10^{-6}	750,000	0.0213	2.13%	4
Herculase® II	1.33×10^{-6}	750,000	0.0213	2.13%	4
Easy-A®	1.33×10^{-6}	750,000	0.0213	2.13%	4
Herculase®	2.67×10^{-6}	375,000	0.0427	4.27%	9
EXL® DNA Polymerase	2.67×10^{-6}	375,000	0.0427	4.27%	9
PicoMaxx®	4.00×10^{-6}	250,000	0.0640	6.40%	13
Taq	8.00×10^{-6}	125,000	0.1280	12.80%	26

* The following assumptions were made to provide the relative mutation frequencies of each DNA polymerase: 1 kb amplicon, 0.1 ng initial target DNA concentration, 30 cycles of PCR, and 16 amplicon doublings.[3]

COMPA

RT-PCR Re
AccuScrip
AccuScrip
AccuScrip
AffinityScr
AffinityScr
AffinityScr *plus* PicoM
AffinityScr *plus* Hercu

"–" indicates

Arc
Polymera

Yield	Speed	Target Length	Blunt or 3′-A ends	Exclusive ArchaeMaxx® Advantage	HotStart Available	Master Mix Available
★★★	★★★★★	0-19 kb (genomic) 0-20 kb (vector) 0-9.6 kb (cDNA)	Blunt	+	+	
★★	★	0-17 kb (genomic) 0-20 kb (vector) 0-9.6 kb (cDNA)	Blunt	+	+	+
★★	★	0-19 kb (genomic) 0-20 kb (vector) 0-9.6 kb (cDNA)	Blunt	+	+	+
★	★	0-10 kb	Blunt			
★★★★★	★★★★★	0-12 kb (genomic) 0-15 kb (vector) 0-7.6 kb (cDNA)	Blunt	+		
★★	★	0-5 kb	3′-A	+	+	+
★★★	★	20-37 kb (genomic) 20-50 kb (vector)	Mixed	+		
★★★★	★	0-10 kb 0-9.6 kb (cDNA)	Mixed	+	+	+

RISON OF OUR RT-PCR KITS AND REAGENTS

eagent	Target Length	Non-Specific Activity	Endonuclease Activity	cDNA Synthesis Attribute	One-Tube RT-PCR Format
t™ High-Fidelity Reverse Transcriptase[g]	0-20 kb	–	–	High Fidelity	
t™ High-Fidelity cDNA Synthesis Kit[g]	0-20 kb	–	–	High Fidelity	
t™ High-Fidelity RT-PCR Kit[g]	0-9 kb	–	–	High Fidelity	
ript™ Multiple Temperature Reverse Transcriptase[g]	0-20 kb	–	–	High Yield at Multiple Temperatures	
ript™ Multiple Temperature cDNA Synthesis Kit[g]	0-20 kb	–	–	High Yield at Multiple Temperatures	
ript™ Multiple Temperature cDNA Synthesis Kit Maxx® High-Fidelity PCR System[e,g]	0-5 kb	–	–	High Yield Sensitivity	
ript™ Multiple Temperature cDNA Synthesis Kit ulase® II Enzyme[a,g]	0-7.6 kb	–	–	High Yield GC-Rich Targets	+

s tested to confirm absence

haeMaxx®
se-Enhancing Factor

The ArchaeMaxx® factor improves the performance of our *Pfu*-based DNA polymerases by overcoming dUTP poisoning.[2]

High-Fidelity Fusion DNA Polymerases

A New Generation of Fast, High-Fidelity DNA Polymerases

Our new generation of high-fidelity fusion DNA polymerases features improved processivity and, with our exclusive enzyme improvement additives, provides superior yield, better reliability, and super-fast cycling times. With the demanding needs of your cloning, site-directed mutagenesis, and expression applications, our high-fidelity fusion DNA polymerases provide you with unsurpassed performance.

A New Standard in High Fidelity PCR Performance

Our high-fidelity fusion DNA polymerases set a new standard in high-fidelity PCR performance. We have upgraded the processivity of our *Pfu*-based enzymes by fusing each DNA polymerase with a high affinity double-stranded DNA binding domain. This domain serves to better anchor the DNA polymerase, preventing early dissociation from the DNA template. The enhanced processivity and exclusive PCR performance additives of our high fidelity fusion DNA polymerases provide you with superior yield, excellent reliability and much shorter overall run times.

We offer two new high-fidelity fusion DNA polymerases with different key features:

- The *PfuUltra*® II Fusion HS DNA Polymerase for ultra-high fidelity
- The Herculase® II Fusion DNA Polymerase for superior yields and GC-rich targets

Enhanced Processivity and Improved Template Integrity

The processivity of our *PfuUltra*® II Fusion HS DNA Polymerase has improved more than 12-fold over the *Pfu* DNA polymerase (Table 1). The *PfuUltra* II enzyme is also shown to be over 5-fold more processive than other fusion enzymes including Phusion™, iProof™, and Pfx50™. This improved processivity of our *PfuUltra* II and Herculase II enzymes allows them to incorporate more nucleotides per binding event, enhancing PCR yields and shortening extension times, hence saving time and improving template integrity by minimizing exposure to extreme cycling temperatures.

Extreme Speed

The enhanced processivity of our *PfuUltra* II and Herculase II high-fidelity fusion DNA polymerases promotes much shorter extension times and more robust, high yield amplifications. While the typical proofreading polymerases require extension times of 1-2 minutes per kilobase, depending upon the target length, *PfuUltra* II and Herculase II fusion DNA polymerases perform exceptionally well with short extension times in "seconds per kilobase." For example, the Herculase II fusion DNA polymerase produced superior yield of a 900-bp human α1AT gene fragment with 1-second extension times (Figure 1).

Enzyme	Median Processivity (nt)	Processivity vs. *Pfu* (fold)
PfuUltra® II Fusion HS DNA Polymerase	185	12.3
Phusion™ DNA Polymerase	30 - 35	2.3
Pfx50™ DNA Polymerase	35	2.3
Cloned *Pfu* DNA Polymerase	15	1.0

Table 1
SUPERIOR PROCESSIVITY OF OUR *PFUULTRA*® II FUSION HS DNA POLYMERASE
Processivity of each enzyme was assayed with each manufacturer's recommended buffer.

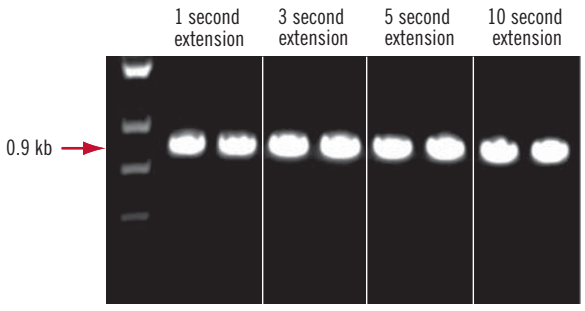

Figure 1
DRAMATICALLY REDUCED EXTENSION TIME
Our Herculase® II Fusion DNA Polymerase amplified a 900-bp human α1AT gene fragment from genomic DNA with extension times as short as one second per cycle.

Ultra-High Fidelity and Fast PCR

PfuUltra® II Fusion HS DNA Polymerase

We are the leading developer of high fidelity PCR enzymes. In our *PfuUltra*® II Fusion HS DNA Polymerase, we coupled polymerase fusion technology with our engineered *PfuUltra* high-fidelity DNA polymerase, hotstart antibody, and proprietary ArchaeMaxx® Polymerase Enhancing Factor. This provides you with extreme accuracy, high specificity, and long target-length capability, while dramatically reducing your overall PCR extension times.

Industry-Leading Fidelity

Our *PfuUltra* II fusion HS DNA polymerase is the new industry standard for ultra-high fidelity PCR. Our data demonstrate that the *PfuUltra* II fusion HS DNA polymerase exhibits ≥3-fold higher accuracy than any other high fidelity enzyme, making it the most accurate enzyme available (Figure 2).

Amplify Long Targets in a Fraction of the Time

The *PfuUltra* II fusion HS DNA polymerase produces higher yields under fast cycling conditions. It amplifies genomic targets up to 19 kb in length (Figure 3). In contrast, most other commercially available fusion enzymes exhibit limited target-length capability, such as up to 6 kb. Using high fidelity enzymes for amplifying long targets is critical since mutation frequency increases linearly with amplicon size.

PCR Enzyme	Accuracy	Target Length	Blunt or 3'-A Ends	ArchaeMaxx® Advantage	Hot Start Available
PfuUltra® II Fusion HS DNA Polymerase	★★★★★	0-19 kb (genomic) 0-20 kb (vector) 0-9.6 kb (cDNA)	Blunt	+	+

High Specificity Hotstart Version

Unlike other commercially available fusion DNA polymerases, our *PfuUltra* II DNA polymerase is formulated with hotstart (HS) antibodies that neutralize both polymerase and exonuclease activities during reaction setup, thereby enhancing specificity and facilitating robotic assemblies.

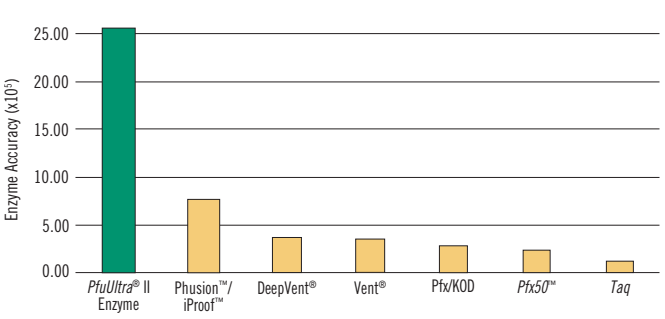

Figure 2
***PfuUltra*® II FUSION HS DNA POLYMERASE, THE HIGHEST FIDELITY PCR ENZYME**
Our *PfuUltra*® II Fusion HS DNA Polymerase exhibits accuracy more than 3-fold higher than the Phusion™ and iProof™ DNA polymerases and 20-fold higher than *Taq* DNA Polymerase. Fidelity was measured using our validated and referenced fidelity assay[5]. (Accuracy = 1/error rate.)

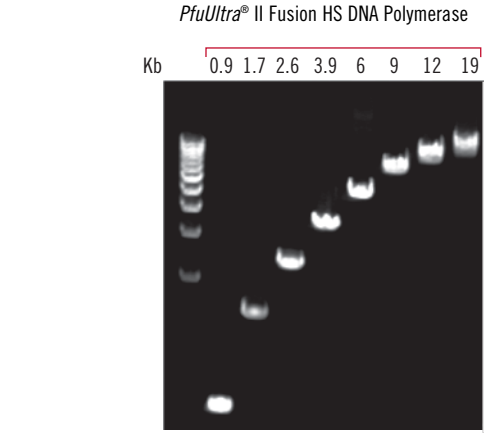

Figure 3
THE *PfuUltra*® II FUSION HS DNA POLYMERASE AMPLIFIES A WIDE RANGE OF TARGETS
Our *PfuUltra*® II Fusion HS DNA Polymerase amplifies a broad range of genomic DNA targets up to 19 kb in length.

Superior Yield, GC-Rich Targets, and High Fidelity

Herculase® II Fusion DNA Polymerase

Our Herculase® II Fusion DNA Polymerase provides accuracy comparable to *Pfu* DNA polymerase and provides superior yield and fast cycling times for routine PCR amplification. Our Herculase® II Fusion DNA Polymerase also helps you overcome challenging PCR with successful amplification of complex and GC-rich templates using a unique buffer system. In addition, Herculase® II Fusion DNA Polymerase is extremely economical.

PCR Enzyme	Accuracy	Target Length	Blunt or 3'-A Ends	ArchaeMaxx® Advantage
Herculase® II Fusion DNA Polymerase	★★★	0-12 kb (genomic) 0-15 kb (vector) 0-7.6 kb (cDNA)	Blunt	+

Superior Yield for Routine Applications

Our Herculase II fusion DNA polymerase is more robust than other PCR enzymes because of its special enzyme formulation and our exclusive ArchaeMaxx® factor. In amplifications of mid-length (1.7 kb) or long (6 kb) genomic DNA fragments, our Herculase II fusion DNA polymerase produced superior yields with extension times as short as 15 sec/kb. With 15 sec/kb extension times, other fast or high-yield proofreading DNA polymerases failed to amplify or produced lower yield of a 6 kb genomic DNA target (Figure 4).

Difficult/GC-Rich Targets

The enhanced processivity of our Herculase II fusion DNA polymerase, the exclusive enzyme improvement additives, and optimized buffer system enable the enzyme to amplify difficult templates such as GC-rich targets. It easily amplifies targets with as high as 84% GC content (Figure 5). DMSO is provided separately as a PCR adjunct, and can be added when amplifying some difficult targets.

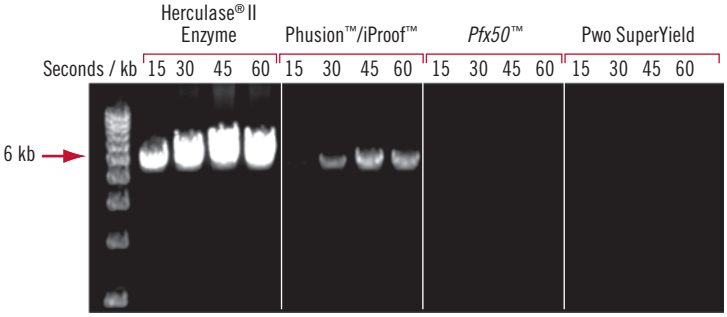

Figure 4
ROBUST YIELDS ACHIEVED WITH FAST CYCLING TIMES
Our Herculase® II Fusion DNA Polymerase produced superior yields of a 6 kb human genomic DNA fragment. Extension times of 15, 30, 45, and 60 sec/kb were employed. Experiments were conducted under identical cycling conditions using each enzyme's recommended buffer and unit concentration.

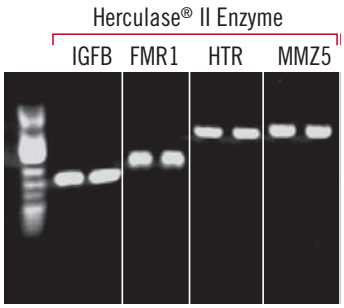

Figure 5
THE HERCULASE® II FUSION DNA POLYMERASE EXCELS IN AMPLIFYING GC-RICH TARGETS
Our Herculase® II Fusion DNA Polymerase easily amplifies GC-rich fragments from human genomic DNA: IGFB, human insulin-like growth factor (79% GC); FMR1, Fragile X mental retardation syndrome protein (84% GC); HTR, hydroxytryptomine receptor C2 frament (65% GC); MMZ5, AIC5- zinc family member 5 protein (68% GC).

Ultra-High Fidelity PCR

Our High-Fidelity PCR Enzyme is Now Available in Convenient Master Mix and Hot Start Formats

Our continuing efforts to improve the fidelity of DNA polymerases resulted in the creation of our *PfuUltra*® High-Fidelity DNA Polymerase. Our *PfuUltra* DNA polymerase is ideal for PCR cloning, site-directed mutagenesis, and whenever sequence accuracy is critical for downstream applications.

Ultra High Fidelity

Our *PfuUltra*® High-Fidelity DNA Polymerase contains a genetically engineered mutant of *Pfu* DNA polymerase that delivers 300% greater accuracy than *Pfu*. A validated and referenced fidelity assay[5] demonstrates that the *PfuUltra* high-fidelity DNA polymerase exhibits an average error rate of only 1 error every 2.5 million bases, 3-fold lower than *Pfu* DNA polymerase and 18-fold lower than *Taq* DNA polymerase (Figure 2).

Enhanced Performance

In addition to ultra-high fidelity, our *PfuUltra* DNA polymerase exhibits superior PCR performance compared to most PCR proofreading enzymes. The addition of our ArchaeMaxx polymerase-enhancing factor promotes higher yields, shorter extension times, and greater target length capability (Figure 6).

PCR Enzyme	Accuracy	Target Length	Blunt or 3'-A Ends	ArchaeMaxx® Advantage	Hot Start Available	Master Mix Available
PfuUltra® High-Fidelity DNA Polymerase	★★★★	0-17 kb (genomic) 0-15 kb (vector)	Blunt	+	+	+

High Specificity Hotstart Version

Our antibody-mediated hotstart formulation, of the *PfuUltra* enzyme and *PfuUltra*® Hotstart DNA Polymerase, provides higher specificity and reduced background than the original formulation.

Convenient Master Mix Formulation

For ultra-high fidelity with added convenience and increased throughput, choose our *PfuUltra*® Hotstart PCR Master Mix[a]. This 2X formulation contains our *PfuUltra* hotstart DNA polymerase, buffer, magnesium, and dNTPs all in one tube for faster setup and more consistent results.

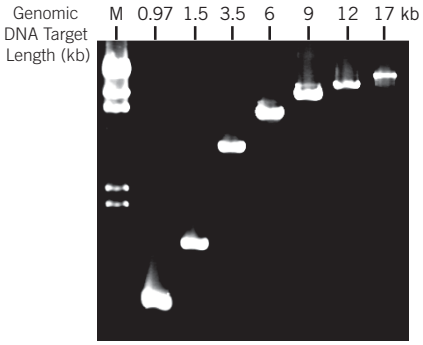

Figure 6
AMPLIFIES A WIDE RANGE OF TARGETS
Our *PfuUltra*® High-Fidelity DNA Polymerase amplifies genomic DNA targets up to 17 kb.

High Fidelity for TA/UA Cloning

Unique Proofreading Formulation Adds A-Overhangs

Since TA or UA cloning requires PCR products with 3′-A overhangs, we created the Easy-A® High-Fidelity PCR Cloning Enzyme to automatically add A-overhangs for quick and easy high fidelity cloning into TA/UA vectors.

PCR Enzyme	Accuracy	Target Length	Blunt or 3'-A Ends	ArchaeMaxx® Advantage	Hot Start Format	Master Mix Available
Easy-A® High-Fidelity PCR Cloning Enzyme	★★★	0-5 kb	3'-A	+	+	+

Combines Cloning Efficiency with High Accuracy

Our Easy-A® High-Fidelity PCR Cloning Enzyme is the only proofreading DNA polymerase formulation to deliver PCR products ready for high-throughput TA or UA cloning. PCR products amplified with the Easy-A PCR cloning enzyme can be cloned directly into the StrataClone™ PCR Cloning Vector with high cloning efficiency and throughput (Figure 7). But unlike *Taq* DNA polymerase, the Easy-A PCR cloning enzyme exhibits proofreading activity that provides the accuracy level of *Pfu* DNA polymerase.

Fidelity Without Extra Work

Adapting blunt-ended fragments amplified with proofreading enzymes requires post-PCR addition of 3′-A overhangs prior to the cloning step. This additional step includes a secondary incubation with *Taq* DNA polymerase and requires the opening and closing of tubes, which exposes the lab to contamination risk. Using our Easy-A high-fidelity PCR enzyme for TA/UA cloning does not require any post-PCR A-addition steps.

High Specificity Hotstart Formulation

The Easy-A high-fidelity PCR cloning enzyme is provided in an antibody-mediated hotstart formulation, providing higher specificity and reduced background.

Convenient Master Mix Formulation

For the highest throughput in TA or UA cloning, choose our Easy-A® High-Fidelity PCR Master Mix. This 2X formulation contains the Easy-A PCR cloning enzyme, buffer, magnesium, and dNTPs all in one tube for faster setup and more consistent results.

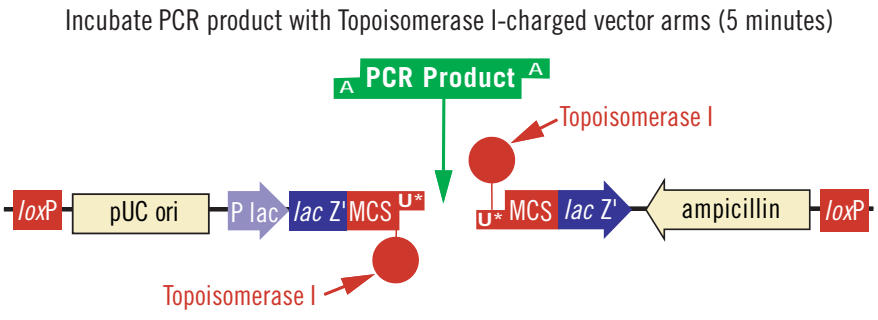

Figure 7
HIGH CLONING EFFICIENCY WITH THE EASY-A® PCR CLONING ENZYME AND THE STRATACLONE™ PCR CLONING KIT
Targets amplified with our Easy-A® High Fidelity PCR Cloning Enzyme contain 3′-A overhangs, allowing quick and easy cloning into our StrataClone™ PCR cloning vector with DNA Topoisomerase I technology.

Specialized High Fidelity DNA Polymerases

Accuracy with Enhanced Performance

We invented the high-fidelity PCR market over a decade ago with the original *Pfu* DNA Polymerase. Since that time, our expertise has led to a number of improvements in high-fidelity performance, including the *PfuTurbo*® DNA Polymerase and the *PfuTurbo*® C_x Hotstart DNA Polymerase[e]. These improvements have made high fidelity PCR more reliable and versatile.

Robust High-Fidelity Amplification

Our *PfuTurbo*® DNA Polymerase is a special formulation of cloned *Pfu* DNA polymerase and our ArchaeMaxx polymerase-enhancing factor. This formulation produces increased PCR product yields without affecting fidelity. The enhanced performance of our *PfuTurbo* DNA polymerase allows for the amplification of longer targets, and the use of lower concentrations of DNA template with shorter extension times than are suitable for *Pfu* DNA polymerase.

ArchaeMaxx® Factor Advantage

Our exclusive ArchaeMaxx factor improves the yield of products, amplified with *Pfu* DNA polymerase by overcoming dUTP poisoning, which is caused by dUTP buildup during PCR through dCTP deamination.[4] The ArchaeMaxx factor prevents the accumulation of dUTP, resulting in greatly enhanced performance over standard archael (proofreading) polymerases.

High Specificity Hotstart Version

The *PfuTurbo*® Hotstart DNA Polymerase is an antibody-mediated hotstart formulation of our *PfuTurbo* DNA polymerase that provides higher specificity, reduced background, and enhanced yield.

PCR Enzyme	Accuracy	Target Length	Blunt or 3'-A Ends	ArchaeMaxx® Advantage	Hot Start Available	Master Mix Available
PfuTurbo® DNA Polymerase	★★★	0-19 kb (genomic) 0-15 kb (vector)	Blunt	+	+	+
PfuTurbo® C_x Hotstart DNA Polymerase	★★★	0-10 kb	Blunt		+	
Pfu DNA Polymerase, cloned or native	★★★	0-5 kb	Blunt			

Novel Mutant Overcomes Uracil Sensitivity

Excessive heat can cause deamination of cytosine residues, resulting in their conversion to uracil. These uracil residues can lead to premature termination of proof reading enzyme activity.[6] We formulated the *PfuTurbo*® C_x Hotstart DNA Polymerase with a novel mutant of *Pfu* DNA polymerase that can read through a uracil located in the template strand without stalling. Thus, the *PfuTurbo* C_x hotstart DNA polymerase improves the overall reliability of high fidelity PCR and exhibits more robust performance (Figure 8).

Prevent Carryover Contamination

Because our *PfuTurbo* C_x hotstart DNA polymerase can replicate uracil-containing DNA, it can be used to prevent carryover contamination. This method involves the use of dUTP in place of dTTP in the nucleotide pool. Subsequent PCR reactions are pre-treated with Uracil-N-glycosylase (UNG) to degrade any dU-containing DNA left over from a previous reaction; the UNG is then inactivated during the next denaturation step. The *PfuTurbo* C_x hotstart DNA polymerase incorporates dUTP, in targets up to 6 kb in length, more efficiently than *Taq* DNA polymerase.

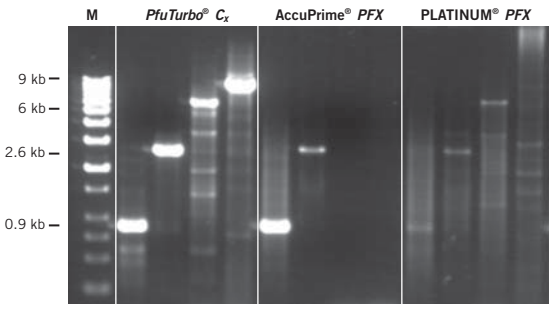

Figure 8
SUPERIOR PERFORMANCE WITH THE *PFUTURBO*® C_X HOTSTART DNA POLYMERASE
Amplification of a range of targets from 0.9 to 9 kb using our *PfuTurbo*® C_x Hotstart DNA Polymerase and other commercially available proofreading PCR enzymes.

DNA Polymerase Blends

Great Performance for Sensitivity, Reliability, and Length

Blending *Taq* DNA polymerase with a proofreading enzyme improves target-length capability, fidelity, and yield. We have developed the PicoMaxx® High-Fidelity PCR System to deliver the highest sensitivity and efficiency at a fraction of the cost of other enzyme blends. To amplify very long targets, our EXL® DNA Polymerase is specially optimized to successfully and accurately amplify long targets up to 50 kb.

PCR Enzyme	Accuracy	Target Length	Blunt or 3'-A Ends	ArchaeMaxx® Advantage	Hot Start Format	Master Mix Available
PicoMaxx® High-Fidelity PCR System	★★	0-10 kb	Mixed	+	+	+
EXL® DNA Polymerase	★★	20-37 kb (genomic) 20-50 kb (vector)	Mixed	+		

Sensitivity and Reliability

Our PicoMaxx® High-Fidelity PCR System is designed to provide superior PCR sensitivity and reliability. Sensitivity is critical for successful amplification, especially with limited or precious samples; use of a PCR enzyme that lacks sensitivity often results in amplification failures. Formulated with a blend of *Taq* and *Pfu* DNA polymerases, our ArchaeMaxx® polymerase-enhancing factor, and a specially optimized buffer, the PicoMaxx high fidelity PCR system overcomes such PCR failures with greater sensitivity than other PCR enzymes (Figure 9).

Convenient Master Mix Formulation

For superior sensitivity and yield with added convenience, choose our PicoMaxx® High Fidelity PCR Master Mix. This 2X formulation contains the PicoMaxx high-fidelity enzyme blend, buffer, magnesium, and dNTPs all in one tube for faster setup and more consistent results.

Tackle Extra Long Amplicons

The EXL® DNA Polymerase provides superior performance in amplifying complex targets greater than 20 kb in length. The EXL DNA polymerase is a special formulation of *Pfu* DNA polymerase, *Taq* DNA polymerase, and our ArchaeMaxx polymerase-enhancing factor, along with a special buffer optimized specifically for very long targets. This enzyme blend provides robust yields and reliable amplification for extra long templates (Figure 10).

Amplify with Higher Accuracy

Because PCR enzymes have an intrinsic rate at which they incorporate incorrect bases, long templates are particularly prone to errors when amplified during PCR. Most commercially available "long & accurate" PCR enzyme blends provide only a slight increase in accuracy over *Taq*. Our EXL DNA polymerase provides twice the accuracy of these other blends to ensure minimal errors in your extremely long PCR products.

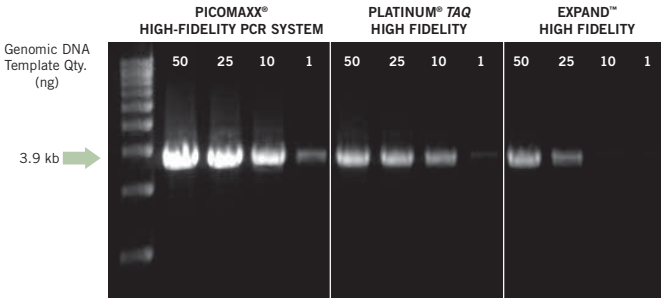

Figure 9
THE PICOMAXX® HIGH-FIDELITY PCR SYSTEM EXHIBITS SUPERIOR SENSITIVITY
We amplified a 3.9-kb α-1 anti-trypsin template with our PicoMaxx® High-Fidelity PCR System, Expand™ High Fidelity PCR System (Roche), and Platinum® *Taq* DNA Polymerase High Fidelity (Invitrogen). We performed all reactions according to the manufacturers' recommendations.

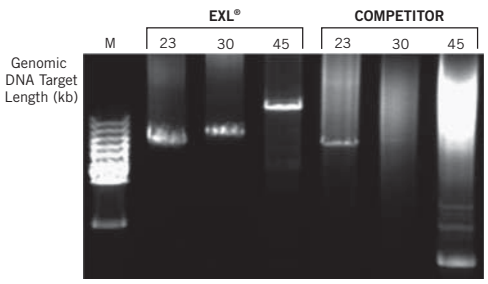

Figure 10
THE EXL® DNA POLYMERASE EXCELS AT AMPLIFYING EXTREMELY LONG TARGETS
The EXL® DNA Polymerase easily amplifies two extremely long genomic targets (23 and 30 kb) and a lambda target (45 kb). A competitor's PCR enzyme for long targets is shown for comparison. All reactions were performed according to the manufacturers' recommendations.

Routine PCR

Economic Alternative to *Taq* DNA Polymerase

Our Paq5000™ DNA Polymerase is a robust *Taq* alternative, developed with a low cost to help you stretch your research dollars. This enzyme provides equivalent and often better performance than *Taq* and can be directly substituted into many *Taq*-based protocols.

Robust PCR with High Yields

Our Paq5000™ DNA Polymerase is designed to provide high yields for end-point PCR applications. Paq5000 DNA polymerase is capable of quickly and robustly amplifying a wide range of DNA target lengths (Figure 11), including genomic targets up to 6 kb in length (Figure 12). In comparison, other standard *Taq* DNA polymerases, given twice the extension time per cycle, did not amplify the same range of DNA targets as strongly (Figure 11).

Reduced Cycling Time

To save you time and increase your PCR yield and throughput, our Paq5000 DNA polymerase is provided with a pre-optimized reaction buffer. Consequently, the extension time per cycle can be shortened to 30 sec/kb instead of the standard one min/kb for *Taq* DNA polymerase.

More Economical than *Taq* DNA Polymerase

The Paq5000 DNA polymerase was developed with the specific intent of providing you with significant cost savings over *Taq*. Please note, this product is not intended for cloning purposes or 5′ nuclease assays. However, our Paq5000 DNA polymerase is ideal for routine PCR applications such as genomic DNA screening and PCR genotyping.

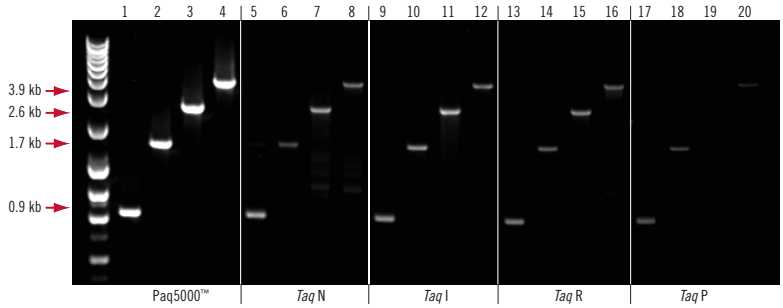

Figure 11
HIGH YIELDS WITH A WIDE RANGE OF TARGET LENGTHS
Amplification of Human α1 Antitrypsin gene (0.9 kb,1.7 kb, 2.6 kb, 3.9 kb) with Paq5000™ DNA Polymerase with 30 sec/kb extension time per cycle (Lanes 1-4). All other reactions were performed with *Taq* DNA polymerases using manufacturers' recommended conditions with 1 min/kb extension time per cycle (lanes 5-8, Supplier N; lanes 9-12, Supplier I; lanes 13-16, Supplier R; lanes 17-20, Supplier P).

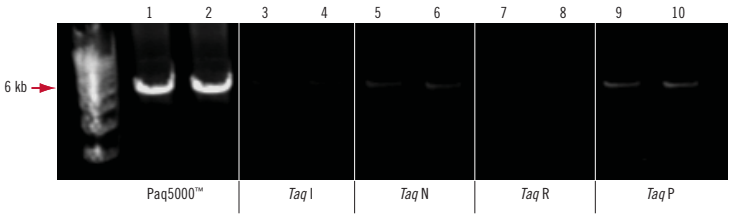

Figure 12
AMPLIFY TARGETS UP TO 6 KB
Amplification of a 6 kb Human ß-Globin target from 100 ng human genomic DNA with Paq5000™ DNA Polymerase with 30 sec/kb extension times (lanes 1 and 2) and *Taq* DNA polymerase from various suppliers with 1 minute/kb extension times (lanes 3-10).

High Fidelity RT-PCR Cloning

Highest Accuracy RT Produces Premium cDNA

Our AccuScript® High-Fidelity Reverse Transcriptase delivers the highest reverse transcription accuracy currently available. This new MMLV-based RT generates cDNA with 3- to 6-fold fewer errors while promoting full-length cDNA synthesis and superior RT-PCR performance specifically for high fidelity cloning, protein expression or sequencing experiments.

RT-PCR Reagent	Target Length	Non-Specific RNase Activity	Endonuclease Activity	cDNA Synthesis Attribute
AccuScript® High-Fidelity Reverse Transcriptase	0-20 kb	-	-	Fidelity 42°C
AccuScript® High-Fidelity 1st-Strand cDNA Synthesis Kit	0-20 kb	-	-	Fidelity 42°C
AccuScript® High-Fidelity RT-PCR Kit	0-9 kb	-	-	Fidelity 42°C

"-" indicates tested to confirm absence

Most Accurate Reverse Transcriptase

Reverse transcriptases (RT) exhibit significantly higher error rates than other known DNA polymerases introducing errors at frequencies of one per 1,500 to 30,000 nucleotides during cDNA synthesis.[1] To resolve the problem of high error rates, we offer the AccuScript® Reverse Transcriptase, which is a Moloney murine leukemia virus reverse transcriptase (MMLV RT) we engineered to deliver the highest transcription accuracy while promoting cDNA synthesis length, yield, and RT-PCR performance. Our AccuScript RT provides over 3- to 6-fold more accurate reverse transcription than other RTs.

Best Overall RT-PCR Accuracy

Combining our AccuScript RT and our *PfuUltra* DNA polymerase lowers the incidence of errors nearly 8-fold compared to identical RT-PCR reactions performed with MMLV RT and a *Taq* high-fidelity blend (Figure 13). Moreover, even when cloning relatively short cDNAs, the probability of recovering error-free clones is dramatically improved by replacing your standard RT-PCR reagents with our AccuScript RT and *PfuUltra* DNA polymerase (Figure 14).

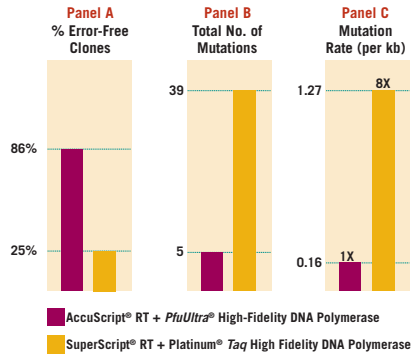

Figure13
EIGHT-FOLD FEWER cDNA ERRORS

We selected and sequenced 30 random clones to determine the number of cDNA clones containing errors (Panel A) and the total number of mutations (Panel B). The number of correct error-free clones (shaded bars in Panel A) using the AccuScript® High-Fidelity Reverse Transcriptase and our *PfuUltra*® High Fidelity DNA Polymerase is 86% vs. only 25% using conventional error-prone RT-PCR reagents. Furthermore, the combination of the AccuScript® RT and our *PfuUltra*® DNA polymerase produced 8-fold fewer errors per kb of cDNA (Panels B and C) compared to a competitor's RT-PCR method.

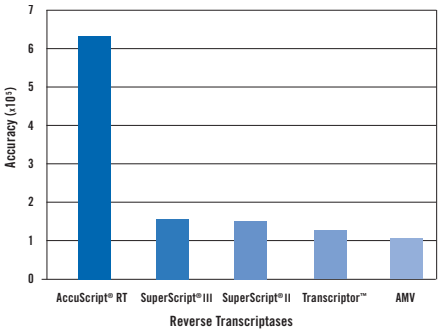

Figure 14
THE ACCUSCRIPT® REVERSE TRANSCRIPTASE FOR ERROR-FREE cDNA

The AccuScript® Reverse Transcriptase delivers greater than 3-fold higher accuracy than other RTs. (Accuracy = 1/error rate)

High Yield Multiple Temperature RT

Achieve High Yields with our Multiple Temperature Reverse Transcriptase

Our new AffinityScript™ Multiple Temperature Reverse Transcriptase (RT) produces high cDNA yields over the broadest temperature range with high affinity to primer:template complexes. It delivers high cDNA yield when amplifying low RNA input amounts and resolving RNA secondary structure using temperatures up to 55°C.

Increased Activity at All Temperatures

Our AffinityScript® Multiple Temperature RT is engineered for improved performance over a broad range of cDNA synthesis temperatures. This multi-temperature capability allows you to change your RT reaction temperature without having to change your reverse transcriptase. You are assured of high cDNA yields from 37°C to 55°C, whether priming at room temperature with random hexamers, or at stringent temperatures designed to enhance priming specificity, or for transcription through GC-rich sequences.

Ideal Sensitivity in Detecting Low Input RNA

When you are working with limited samples, such as small animal models, small organs, or biopsies, our AffinityScript RT is the ideal choice to convert that precious RNA into stable cDNA. Our RT routinely detects and amplifies targets using 1 ng of total RNA.

RT-PCR Reagent	Target Length	Non-Specific RNase Activity	Endonuclease Activity	cDNA Synthesis Attribute	One-Tube RT-PCR Format
AffinityScript™ Multiple Temperature RT	0-20 kb	-	-	Yield 37°- 55°C	
Easy-A® One-Tube RT-PCR System	0-5 kb	-	-	Yield 42°C	+

"-" indicates tested to confirm absence

Two RT-PCR Systems for Cloning or High Sensitivity

Our AffinityScript RT is engineered to produce the highest yields of full length cDNA. Whether you are working with genes as small as 500 to 1000 bases or as large as 5, 10, or even 20 kb, the AffinityScript RT is the ideal choice to convert these RNA transcripts into full length cDNA. To demonstrate full length feature, we PCR amplified a 0.6-kb product located at the 5′ end of the human nebulin mRNA, which is 20 kb in length, indicating complete reverse transcription of the nebulin mRNA.

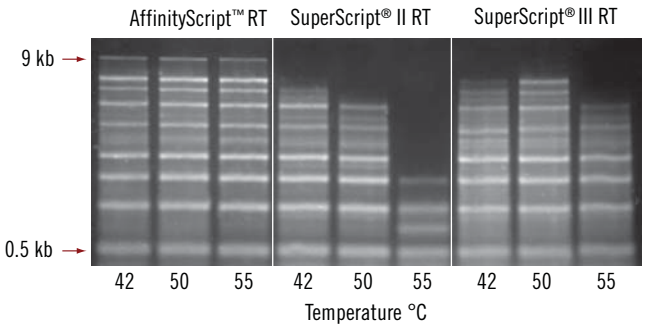

Figure 15
SUPERIOR cDNA YIELDS OVER A BROADER TEMPERATURE RANGE WITH AFFINITYSCRIPT™ MULTIPLE TEMPERATURE REVERSE TRANSCRIPTASE
cDNA was generated from 1 µg of RNA ladder (Ambion) using equivalent amounts of each reverse transcriptase at the indicated temperatures (42, 50, and 55°C). After cDNA synthesis, the samples were resolved on a 1% alkaline agarose gel. The AffinityScript™ Multiple Temperature RT generates significantly more, longer cDNA (up to 9 kb!) than competitor enzymes.

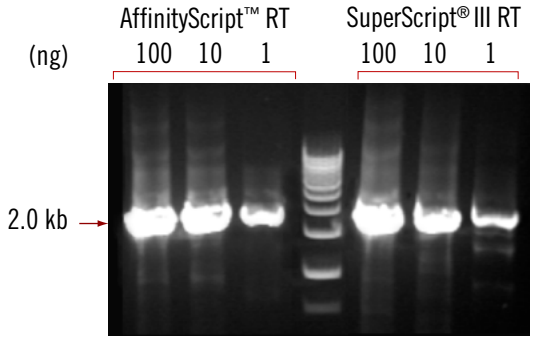

Figure 16
AFFINITYSCRIPT™ RT DELIVERS HIGH YIELD FROM THE LOWEST STARTING AMOUNT OF RNA IN END POINT RT-PCR
cDNA was synthesized from HeLa total RNA using AffinityScript™ RT (42°C) and SuperScript® III RT (50°C) according to manufacturers' instructions; the PicoMaxx® enzyme was used to PCR amplify a 2-kb fragment. The AffinityScript™ RT generated the most cDNA from smallest RNA input vs. SuperScript® III RT (lane 3 vs. lane 10).

High Performance PCR Cloning

StrataClone™ PCR Cloning Kits with DNA Topoisomerase I Technology

Our StrataClone™ PCR Cloning Kit[g] makes cloning your PCR products easier, faster, and more reliable with DNA topoisomerase I technology. Go from PCR to transformation in one short, three-step protocol.

Fast, Easy, Reliable PCR Cloning

Our StrataClone™ PCR Cloning Kits provide the highest-efficiency option for topoisomerase-based PCR cloning. Cloning PCR products using DNA topoisomerase I technology saves you time and money over conventional PCR cloning with simple primer design, no PCR clean-up, and an easy three-step process. Simply add your PCR product to the vector mix, incubate 5 minutes at room temperature, and transform ligated DNA into competent cells.

StrataClone™ Technology

The StrataClone PCR cloning technology exploits the combined efforts of two enzymes, DNA topoisomerase I from *Vaccinia* virus and Cre recombinase from bacteriophage P1. In *Vaccinia*, DNA topoisomerase I assists in DNA replication by relaxing and rejoining DNA strands. The enzyme's site-specific endonuclease activity generates linear DNA with defined termini, and the DNA ligase activity closes the strand when replication is complete. When topoisomerase I cleaves a phosphodiester backbone of the DNA duplex, a covalent DNA-enzyme intermediate is formed, which conserves bond energy to be used for religating the cleaved DNA back to the original strand or to a heterologous DNA acceptor. The site-specific Cre recombinase enzyme catalyzes DNA recombination between asymmetric 8-bp core regions of two *lox*P recognition sequences.

The StrataClone PCR cloning vector mix contains DNA arms charged with topoisomerase I on one end and *lox*P recombination sequences on the other. The topoisomerase-charged ends have a modified uridine overhang for direct ligation of *Taq*-amplified or Easy-A-amplified PCR products. When your PCR product is added to the mix, the DNA topoisomerase I ligates the vector arms to the PCR product resulting in a linear molecule (vector arm–PCR product–vector arm). The time required to ligate amplicon to vector is significantly reduced by the ready availability of the topoisomerase enzyme covalently bound to the vector arm. The ligated DNA is then transformed, with no clean-up steps required, into our high-efficiency competent cell line engineered to transiently express Cre recombinase. The linear DNA is circularized by the Cre recombinase at the *lox*P sites and the recombinant vector is amplified by the host cell in an overnight incubation (Figure 17).

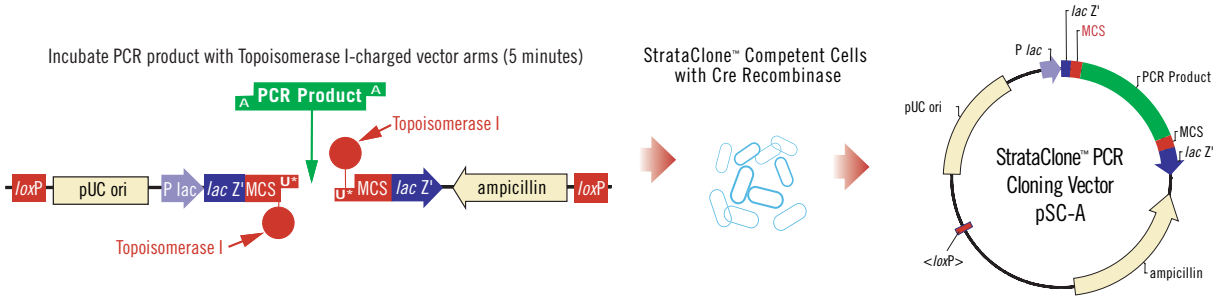

Figure 17
THE STRATACLONE™ PCR CLONING TECHNOLOGY
Our StrataClone™ PCR Cloning System Kits exploit the combined efforts of two enzymes, *Vaccinia* DNA Topoisomerase I and bacteriophage P1 Cre recombinase.

Ordering Information

PCR Enzymes

Product Name	Size	Catalog
Ultra-High Fidelity		
PfuUltra® II Fusion HS DNA Polymerase	40 rxn	600670
	200 rxn	600672
	400 rxn	600674
PfuUltra® High-Fidelity DNA Polymerase	100 U	600380
	500 U	600382
	1000 U	600384
PfuUltra® Hotstart DNA Polymerase	100 U	600390
	500 U	600392
	1000 U	600394
High Fidelity, TA or UA Cloning		
Easy-A® High-Fidelity PCR Cloning Enzyme	100 U	600400
	500 U	600402
	1000 U	600404
High Fidelity, Blunt Cloning		
Pfu DNA Polymerase, Cloned	100 U	600153
	500 U	600154
	1000 U	600159
Pfu DNA Polymerase, Native	100 U	600135
	500 U	600136
	1000 U	600140
PfuTurbo® C_x Hotstart DNA Polymerase	100 U	600410
	500 U	600412
	1000 U	600414
PfuTurbo® DNA Polymerase	100 U	600250
	500 U	600252
	1000 U	600254
PfuTurbo® Hotstart DNA Polymerase	100 U	600320
	500 U	600322
	1000 U	600324
Superior Yield and GC-Rich/Complex Targets		
Herculase® II Fusion DNA Polymerase	40 rxn	600675
	200 rxn	600677
	400 rxn	600679
Herculase® Enhanced DNA Polymerase	100 U	600260
	500 U	600262
	1000 U	600264
Herculase® Hotstart DNA Polymerase	100 U	600310
	500 U	600312
	1000 U	600314
High Sensitivity & Economy		
PicoMaxx® High-Fidelity PCR System	100 U	600420
	500 U	600422
	1000 U	600424
Extra-Long Targets		
EXL® DNA Polymerase	100 U	600340
	500 U	600342
	1000 U	600344

PCR Enzymes, continued

Product Name	Size	Catalog
Routine PCR		
Paq5000™ DNA Polymerase	500 U	600680
	1000 U	600682
	5000 U	600684
SureStart® *Taq* DNA Polymerase[a]	100 U	600280
	500 U	600282
	1000 U	600284
Taq2000™ DNA Polymerase[a]	100 U	600195
	500 U	600196
	1000 U	600197

PCR Master Mixes

Ultra-High Fidelity		
PfuUltra® Hotstart PCR Master Mix	100 rxn	600630
	400 rxn	600632
High Fidelity, TA or UA Cloning		
Easy-A® High-Fidelity PCR Master Mix	100 rxn	600640
	400 rxn	600642
High Fidelity, Blunt Cloning		
PfuTurbo® Hotstart PCR Master Mix	100 rxn	600600
	400 rxn	600602
Superior Yield and GC-Rich/Complex Targets		
Herculase® Hotstart PCR Master Mix	100 rxn	600610
	400 rxn	600612
High Sensitivity & Economy		
PicoMaxx® High-Fidelity PCR Master Mix	100 rxn	600650

RT-PCR Reagents

RT-PCR Reagents		
Accuscript® High-Fidelity 1st Strand cDNA Synthesis Kit	50 rxn	200820
Accuscript® High-Fidelity Reverse Transcriptase	50 rxn	600089
	200 rxn	600090
Accuscript® High-Fidelity RT-PCR Kit	50 rxn	600180
AffinityScript™ Multiple Temperature Reverse Transcriptase	10 rxn	600105
	50 rxn	600107
	200 rxn	600109
AffinityScript™ Multiple Temperature cDNA Synthesis Kit	50 rxn	200436

PCR-Related Kits & Accessories

PCR Cloning Kit		
StrataClone™ PCR Cloning Kit	10 rxn	240206
	20 rxn	240205
Deoxynucleotide Mix	400 µl	200415

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